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Article

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Improving sludge settleability by introducing an innovative, two-stage settling sequencing batch reactor

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Abstract

Sludge settleability is considered one of the main drawbacks of sequencing batch reactors. The aim of this study therefore is to improve sludge settleability by introducing a novel, two-stage settling sequencing batch reactor (TSSBR) separated by an anoxic stage. The performance of the TSSBR was compared with that of a normal operating sequencing batch reactor (NOSBR), operating with the same cycle time. The results show a significant improvement in sludge settleability and nitrogen compound removal rates for the TSSBR over the NOSBR. The average removal efficiencies of NH_3-N, NO_3-N and NO_2-N have been improved from 76.6%, 86.4% and 87.3% respectively for the NOSBR to 89.2%, 95.2% and 96% respectively for the TSSBR. In addition, the average SVI_{30} for the NOSBR has been reduced from 42.04 ml/g to 31.17 ml/g for the TSSBR. After three months of operation, there was an overgrowth of filamentous bacteria inside the NOSBR reactor, while the morphological characteristics of the sludge inside the TSSBR reactor indicated a better and homogenous growth of filamentous bacteria.

Keywords: Filamentous bacteria; Nitrogen removal; Sequencing batch reactor; Sludge settleability; Wastewater treatment.

1. Introduction

The continuing increase in industrial activities worldwide, is having an adverse impact on the environment. If not committed to protective government regulations, industrial plants can discharge highly toxic wastewater consisting of environmentally noxious materials, this constituting a serious threat to both the environment and humans [1]. There are a significant number of technologies available for the treatment of industrial wastewater; biological treatment is no exception. The latter is considered one of the most convenient technologies for the treatment of industrial wastewater due to its manufacturing and operational cost requirements. In addition to cost considerations, biological treatment has proved to be an effective technology for removing high concentrations of pollutants.

One of the common biological technologies is the activated sludge process (ASP), used worldwide for the treatment of domestic and industrial wastewater [2]. It consists of several reactors in which microorganisms degrade incoming wastewater and in doing so, grow and produce new microorganisms. After degradation is achieved, these microorganisms are separated from the treated wastewater by sedimentation. In order to sustain an active and high concentration of solids for the reaction treatment, some sediment solids should be removed from the system, others recycled back into the aeration basin [3]. One of the drawbacks of ASP is that it requires a large footprint for its treatment tanks [4].

Often industries are located within cities which makes it difficult to build a treatment system containing several tanks. In this case, alternatives are available such as sequencing batch reactors (SBR). The SBR is an activated sludge process that consists of a sequence of stages which operate in one tank following a time sequence. These stages are fill, react, settle, draw and idle. It has been reported that SBRs require less area, are flexible to operate and can be operated automatically [5, 6]. However, solid-liquid separation, or sludge bulking, is still one of the most problematic issues with SBRs and ASPs in general [7, 8].

Researchers have reported several reasons for this problem such as difficulty in handling sudden changes in the operating parameters [9, 10], microbial clustering behaviour [11], the overgrowth of filamentous bacteria [12, 13], foaming [14, 15], pin-point sludge [15, 16], poor macrostructure [15], poor flocculation properties [17] and floc size distribution [13, 18].

To overcome settling problems, researchers have been evaluating a variety of solutions, one of which is granulation technology. In specific environments, microbial self-agglomeration forms a granular biological polymer known as aerobic granular sludge (AGS) [19, 20]. It has many advantages such as high degradation abilities, significant settling velocity, a regular shape and compact structure [4, 20]. However, the stability of AGS may decline after extended periods of operation [21, 22]. In addition to stability loss, granulation technology has other problems such as high operation temperatures, a long acclimatisation time and inefficiency when subject to low concentrations of organic wastewater [23, 24]. This means that granulation technology requires more research to address these issues. A different approach to overcoming settling problems is the addition of chemicals before the settling stage to improve the settling performance [25, 26]. However, this procedure raises the cost of treatment and results in more complex and toxic residual which has a negative impact on the environment [27]. Along with granulation sludge technology and chemical conditioning, researchers have been modifying operation strategies and trialling the addition of more stages to SBR treatment cycles to improve the treatment performance without additional costs due to increased cycle time [4, 28, 29].

The aim of this study is to improve sludge settleability by introducing a novel, two-stage settling SBR. This system will focuses on three issues. The first is to create a shock after the first settling stage and allow small flocs to climb together, merge with large flocs and settle again in the second settling stage. Secondly, the effect of this procedure will be assessed to examine the elimination of filamentous accumulation and improvement in the settling stage.

Finally, verification will be sought of whether separating the two stages of settling with a short anoxic stage enhances nitrogen removal efficiency by improving the denitrification stage.

2. Materials and methods

2.1 Activated sludge characteristics and synthetic wastewater

The returned activated sludge (RAS) used in this study was collected from a treatment plant at Sandon Docks, United Utilities, Liverpool, UK. Synthetic wastewater was prepared every week by mixing the chemicals in Table 1 [30, 31] with deionized water. All reagents used in this study were purchased from Sigma-Aldrich, UK.

Table 1: Composition of synthetic wastewater

Chemicals	Chemical formula	Concentration
Glucose	$C_6H_{12}O_6$	500 mg/l
Magnesium Sulphate Heptahydrate	$MgSO_4.7H_2O$	5 mg/l
Sodium Bicarbonate	$NaHCO_3$	200 mg/l
Ammonium Chloride	NH_4Cl	25 mg/l
Potassium Nitrate	KNO_3	25 mg/l
Monobasic Potassium Phosphate	KH_2PO_4	5 mg/l
Iron(III) Chloride Hexahydrate	$FeCl_3.6H_2O$	1.5 mg/l
Calcium Chloride Dihydrate	$CaCl_2.2H_2O$	0.15 mg/l

2.2 Experimental setup and operation of the treatment reactors

Four Plexiglas reactors were used in this study as shown in Fig. 1. The total volume of each reactor is 6.5 litres, 5 litres the working volume of each. For the aeration stage, an air pump was used to supply air at the rate of 1l/m, air diffusers used inside each reactor to produce fine air bubbles. Overhead stirrers were used for each reactor to achieve the anoxic stages. Three electronic sensors (probes) were positioned in each reactor to monitor the pH, oxidation-reduction potential (ORP) and temperature. Dissolved oxygen (DO) was measured manually using a HACH portable meter. A 100 litre storage tank was used to store synthetic wastewater. One reactor was used for the normal operation sequencing batch reactor (NOSBR), a second reactor used as the two-stage settle sequencing batch reactor (TSSBR). The operation cycles for the NOSBR and the TSSBR are shown in Table 2, the time allocated for each cycle was achieved using the same cycle as Chen et al. [32] after optimising the aeration time to find the optimal operation of the system. All the probes and the software used for recording measurements were purchased from Pico Technology, UK.

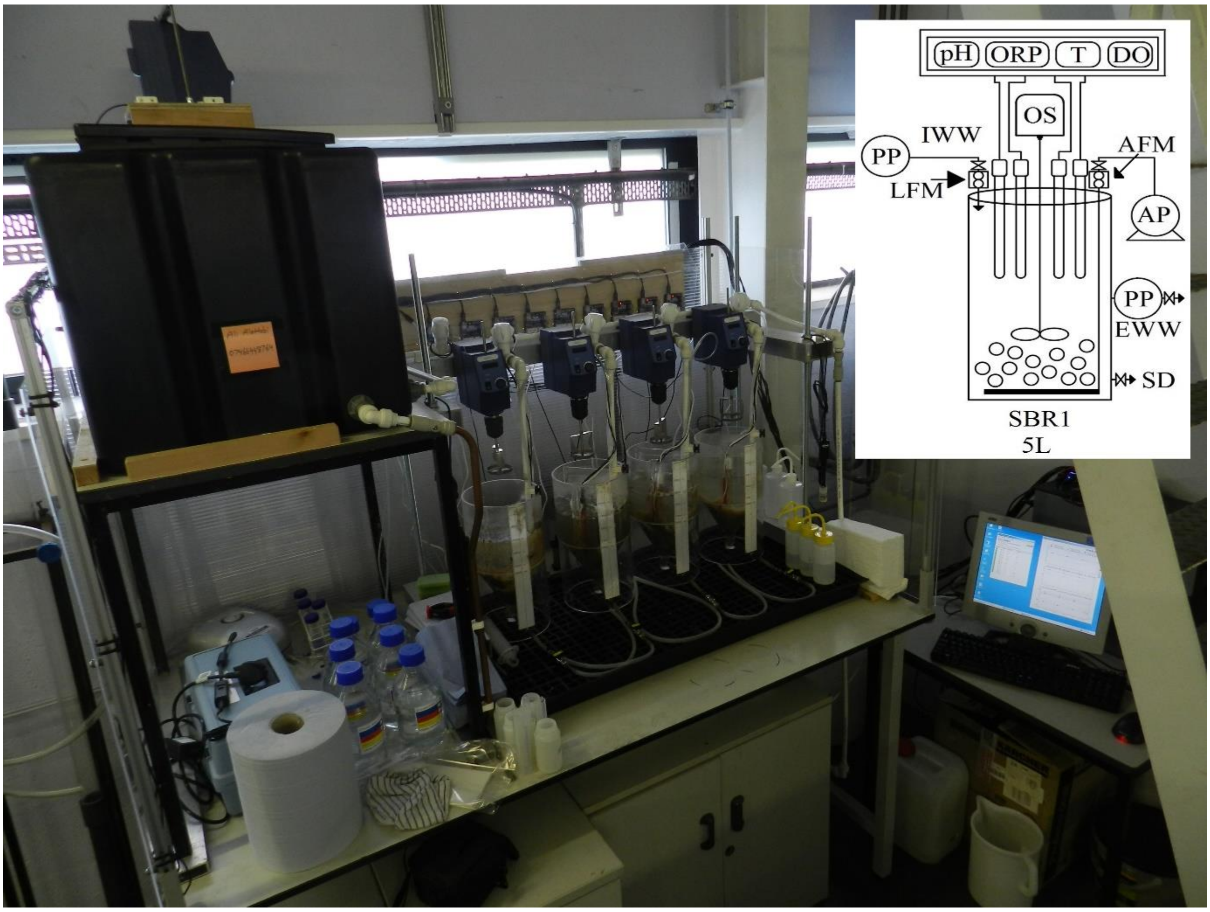

Fig. 1. The configuration of the laboratory-scale SBRs (AFM: air flow meter; AP: air pump; DO: dissolved oxygen probe; IWW: Influent wastewater; EWW: effluent wastewater; LFM: liquid flow meter; ORP: oxidation-reduction potential probe; PP: peristaltic pumps; OS: overhead stirrer; pH: pH probe; SD: sludge draw; T: temperature probe).

Table 2: NOSBR and TSSBR operation cycles

NOSBR 5.5 h	Anoxic Fill	Aerobic React	Settle	Draw & Idle
Time (min)	15	240	60	15

TSSBR 5.5 h	Anoxic Fill	Aerobic React	Settle I	Anoxic mixing	Settle II	Draw & Idle
Time (min)	15	240	15	15	30	15

The treatment reactors were filled with 1.5 litres of RAS and 3.5 litres of synthetic wastewater. The pH of the reactors was maintained between 6.5 and 8.5. The temperature between 6 and 15 ˚C (ambient temperature), this range was taken according to the weather in the UK, where the study was performed; it is the same range for the treatment plant that provides the RAS for this study. The RAS acclimatisation stage lasted 20-30 days. The reactors were operated with between 2500 and 3500 mg/l of MLSS, the MLVSS/MLSS ratio 0.83. The solids retention time (SRT) for both NOSBR and TSSBR was kept at 20 days to achieve the optimal operation for the system. This time was maintained by controlling the waste activated sludge for both systems (NOSBR and TSSBR) at the same rate. Influent and effluent samples were taken from each reactor to study treatment efficiency and settling performance and to compare the results between the NOSBR and TSSBR. The reactors were operated continuously for 3 months, the sampling and analysis performed twice a week.

2.3 Analytical methods

The concentrations of chemical oxygen demand (COD), ammonia-nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), mixed liquor suspended solids (MLSS), settled sludge volume (SSV) and sludge volume index (SVI) for the influent and effluent samples were measured according to standard methods [33], after filtering the samples through 0.45 µm filter paper. COD and nitrogen compounds removal efficiency was tested two days per week over a two-month period. The settling performance was also tested two days a week but over a three-month period.

2.4 Morphological study and image analysis procedure for the sludge

Sludge settling is a critical issue in most treatment plants as it can increase the time needed for treatment which increases operation costs. The sludge volume index is the most common indicator of sludge settleability [34]. SVI has been use widely to test sludge settleability in both laboratory scale and pilot plant scale studies [35]. The settleability of activated sludge systems can also be monitored and controlled through microscopic observation [13, 36]. Quantitative image analysis is a promising technique which has been used to study different problems in activated sludge systems [2, 13, 37, 38]. In this research, SVI_{30} was used to determine settling performance along with a quantitative study for sludge samples which targeted the filamentous bacteria as this is considered one of the main reasons for sludge settling problems as mentioned earlier.

A light microscope (AX10, Zeiss, Germany) with a colour video camera (PixeLINK, Canada), was used to examine the morphological characteristics of the sludge by capturing images and analysing them via image processing software. Over the period of the study, samples were taken from both treatment reactors every other day to record differences in filamentous growth and the diversity of sludge characteristics between the reactors to relate this to sludge settleability. Pictures were taken under 100x magnification. Two microscope slides were used for each sample, and for each slide, 10µL of the sample was poured onto the slide using a micropipette [13]. A total of 80 images were captured for each sample (40 images per slide) to avoid bias. A quantitative study of the captured images was conducted by studying the ratio of total filament length per MLSS value (TL/MLSS), and the ratio of total filament length per sample volume (TL/ Vol). This was achieved using the same method as Mesquita et al. [39]. Image acquisition, background pre-treatment, aggregate segmentation, filamentous segmentation and debris elimination were carried out as shown in Fig. 2, using MATLAB 9 (The Mathworks, Natick, USA), following Mesquita et al.'s [39] procedure.

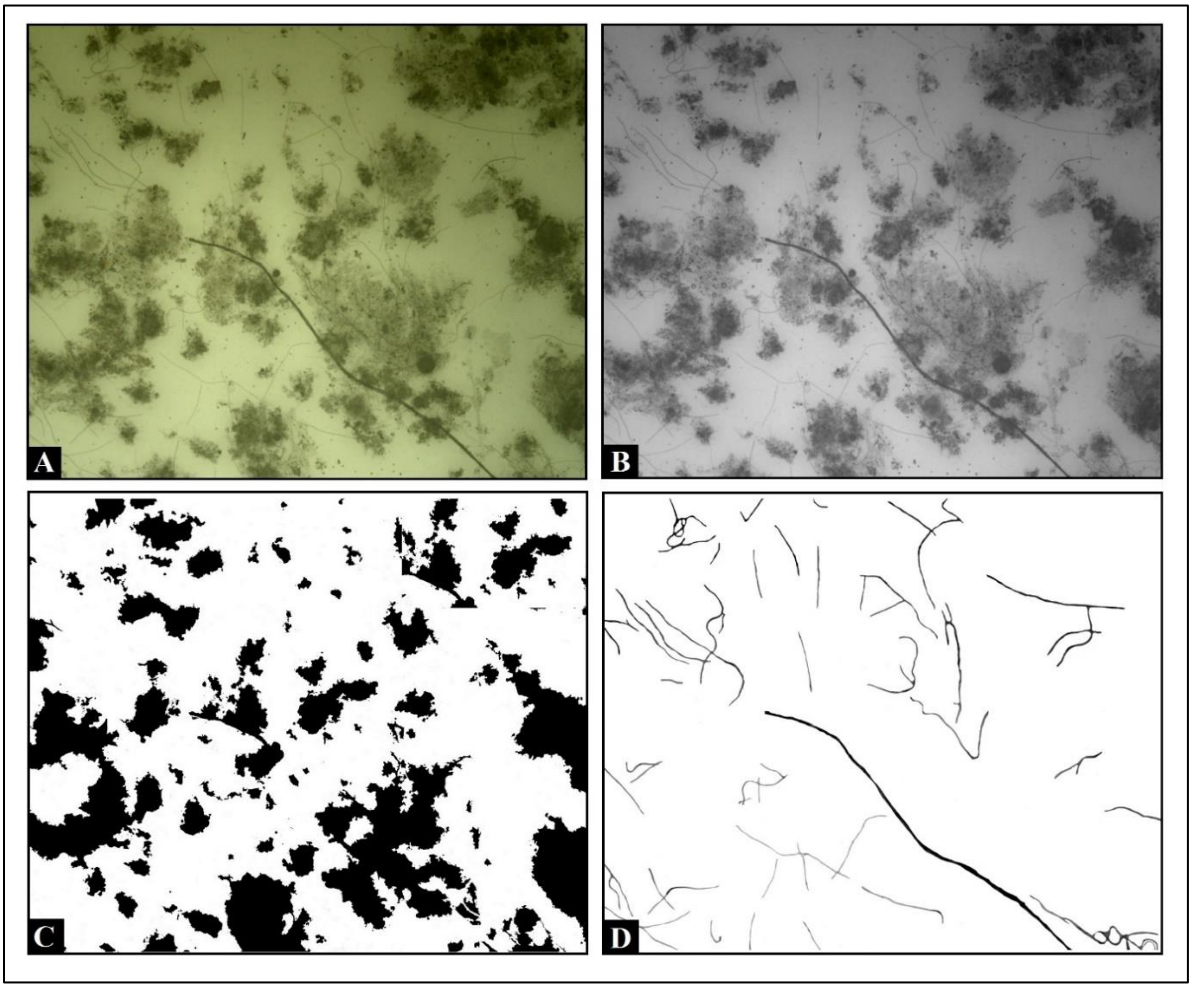

Fig. 2. Schematic representation of the image processing program. (a) Image acquisition, (b) background pre-treatment, (c) aggregate segmentation, and (d) filamentous segmentation and debris elimination.

3. Results and discussion

Two SBR reactors were used to examine the efficiency of the treatment and settling performance; the NOSBR (5.5 h cycle time) and TSSBR (5.5 h cycle time). The cycle strategies for NOSBR and TSSBR are shown in Table 2.

3.1 Efficiency of the removal of COD and nitrogen compounds

The efficiency of the removal of COD for the NOSBR and TSSBR are shown in Fig. 3a. The average efficiency for the removal of COD in the NOSBR and TSSBR were 93.7% and 93.1%, respectively, the average effluent 54.83 mg COD/l and 53.7 mg COD/l, respectively. The similarities in efficiency for both reactors could be due to the same reaction time and operating conditions. The anoxic stage in the TSSBR enhanced the efficiency of the removal of nitrogen compounds as shown in Figs. 3b, 3c and 3d. The average efficiency of removal of NH_3-N, NO_3-N and NO_2-N for the NOSBR was 76.6%, 86.4% and 87.3%, respectively with an average effluent of 1.87 mg NH_3-N/l, 2.41 mg NO_3-N/l and 2.23 mg NO_2-N/l. The average efficiency of the removal of NH_3-N, NO_3-N and NO_2-N for the TSSBR was 89.2%, 95.2% and 96%, respectively with an average effluent of 0.85 mg NH_3-N/l, 0.81mg NO_2-N/l and 0.75 mg NO_2-N/l. The reason for this could be the enhancement of the nitrogen cycle by offering an anoxic stage between the two settling stages in the TSSBR. During the anoxic fill, ammonia can be decreased by half [40], and denitrification might be occurring due to low DO concentrations and the presence of a carbon source. Ammonium was oxidized completely during the aeration stage while the remaining nitrate and nitrite was removed during the second anoxic stage in the TSSBR as shown in Fig. 4. This is the reason why the nitrogen compounds were removed more effectively in the TSSBR in comparison to the NOSBR. These results substantiate the work of Chen et al. [4] who studied a step-feeding SBR and achieved high nitrogen removal rates using two aeration phases. Chen et al. [4] also stated that the anoxic condition during the feeding stage could result in a high rate of denitrification which in turn, leads to high nitrogen removal efficiency.

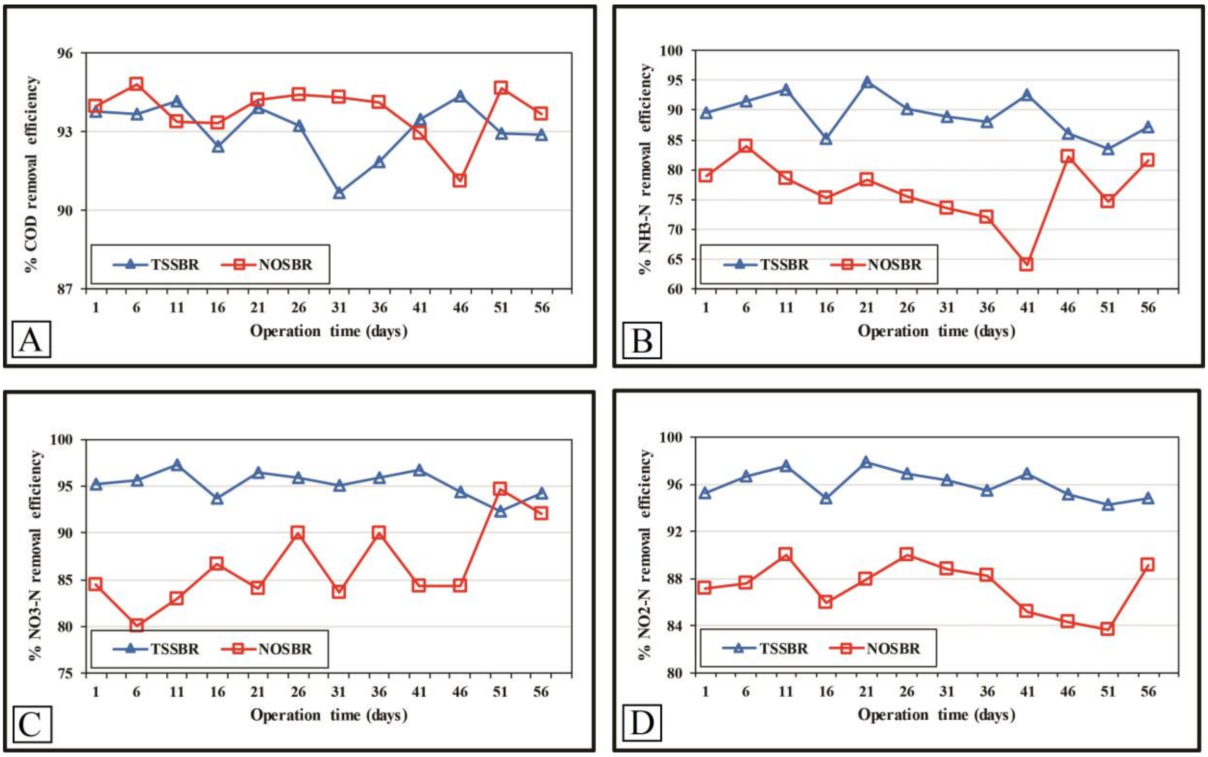

Fig. 3. The removal efficiency of NOSBR and TSSBR for a) COD, b) NH_3-N, c) NO_3-N, d) NO_2-N.

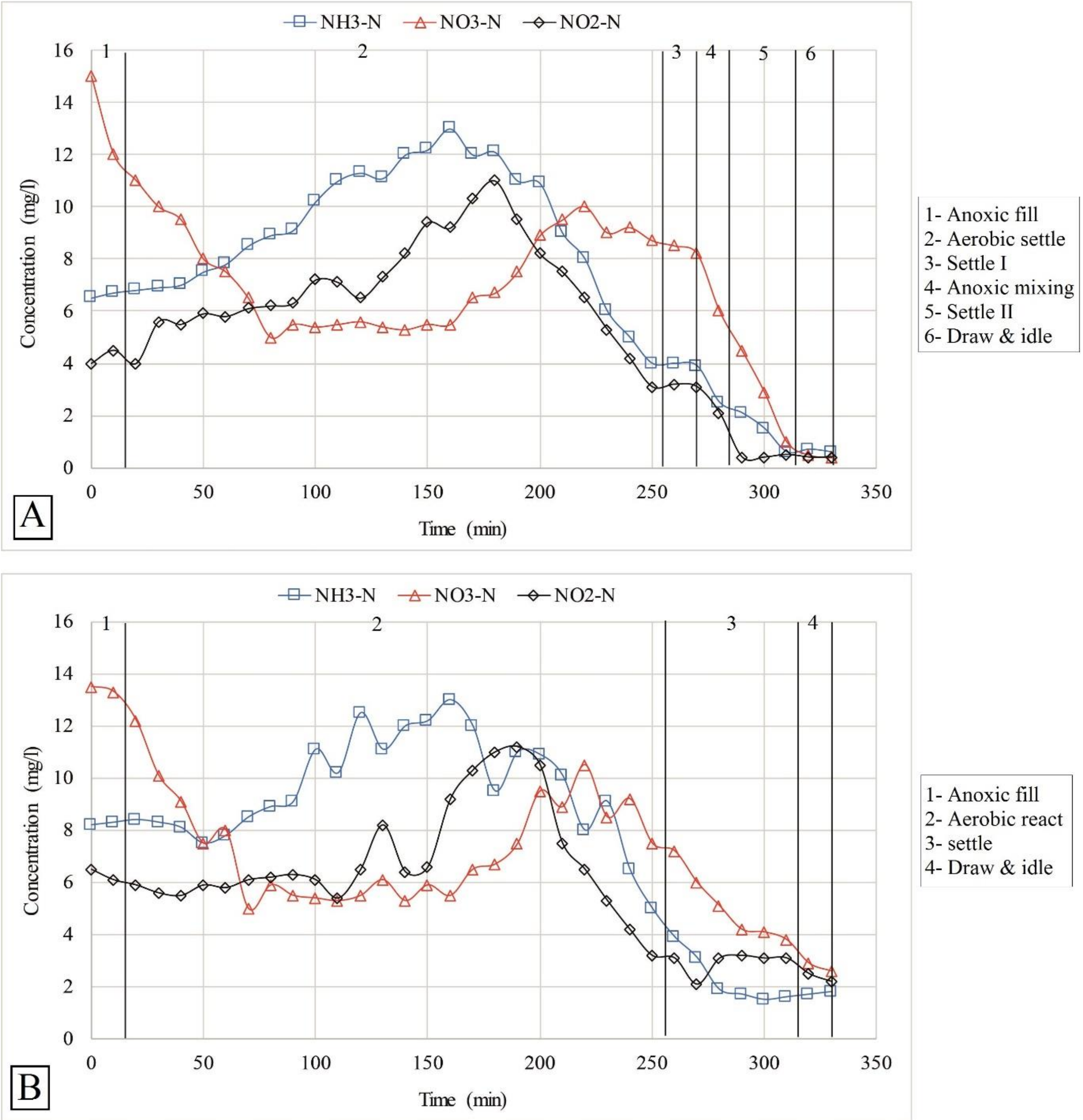

Fig. 4. The concentration of NH_3-N, NO_3-N and NO_2-N during one treatment cycle of a) TSSBR, b) NOSBR

3.2 Solids settling performance

As shown in Figs. 5a and 5b, the settling ability of the TSSBR is clearly better than the NOSBR. The average SVI_{30} for TSSBR and NOSBR was 31.17 ml/g and 42.04 ml/g, respectively. The quantitative microscopic study for filamentous growth reported the same result as shown in Figs. 5c and 5d. The average TL/MLSS for TSSBR and NOSBR were 1475.33 mm/mg and 1594.34 mm/mg, respectively while the average TL/Vol were 139.70 mm/µl and 221.79

mm/μl, respectively. By plotting the results of TL/MLSS and TL/Vol with SVI_{30} results, a highly significant relationship was found as shown in Figs. 5e, 5f, 5g and 5h, which means that the total length of filamentous bacteria affects sludge settleability. Similar findings were reported by Schuler and Jassby [41] and Jassby et al. [2] in that they also found a single linear relationship between SVI and filament content.

During the first month, there was no clear difference between the morphological characteristics of TSSBR and NOSBR, as seen in Fig. 6. However, in the second and third months, the settling ability of the NOSBR dropped due to the filamentous growth inside the reactor as seen in Fig. 6. The morphological characteristics of the sludge inside the TSSBR reactor have better and more homogenous growth of filamentous bacteria as also seen in Fig. 6.

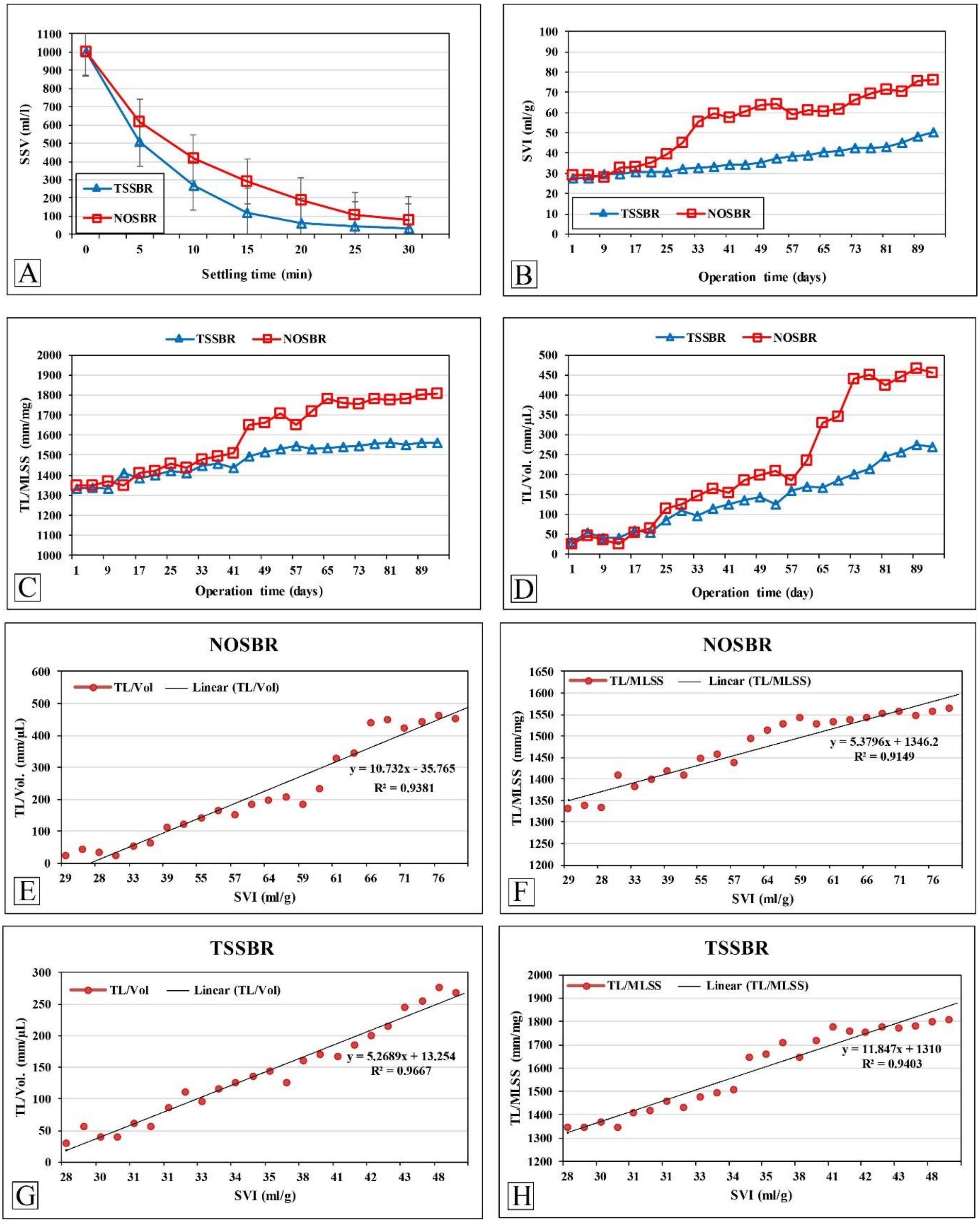

Fig. 5. a) Settled sludge volume for NOSBR and TSSBR, b) Sludge volume index for NOSBR and TSSBR, c) Total filament length per MLSS for NOSBR and TSSBR, d) Total filament length per the sample volume for NOSBR and TSSBR, e) Total filament length per the sample volume vs. SVI for NOSBR, f) Total filament length per MLSS vs. SVI for NOSBR, g) Total filament length per the sample volume vs. SVI for TSSBR, h) Total filament length per MLSS vs. SVI for TSSBR.

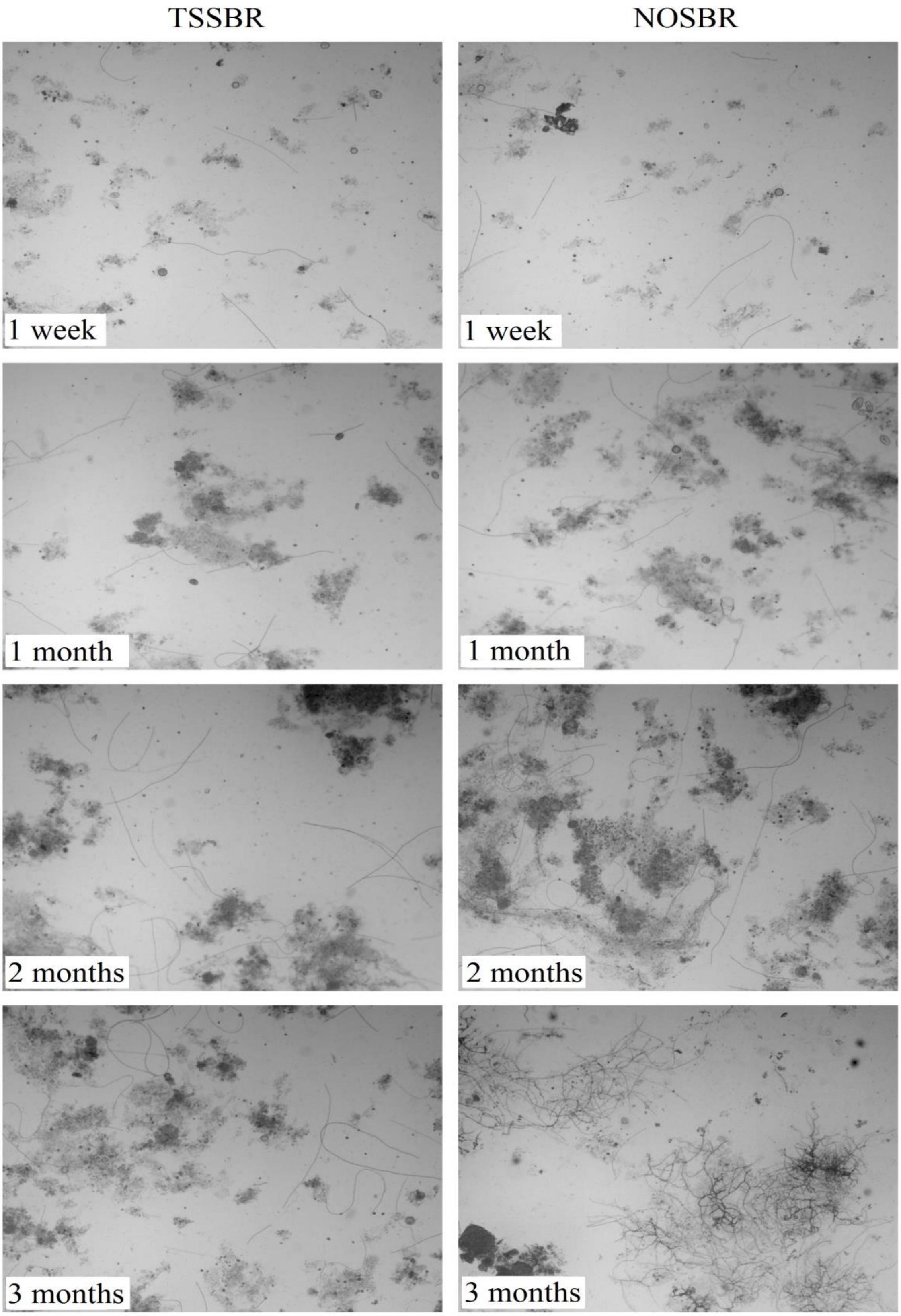

Fig. 6. 100x microscopic images of sludge sample for NOSBR and TSSBR during different ages (1 week, 1 month, 2 months and 3 months).

Over the three-month operation time, the sludge was kept at between 12-18 days of age, the MLSS concentrations between 2500 mg/l and 3500 mg/l for both reactors as seen in Fig. 7. There are two potential reasons for the improvements seen in the TSSBR. The first could be due to breaking down the long settling stage into two stages and producing a shock in the anoxic mixing stage after the first settling stage. This led to a better compaction of settled and non-settled particles as well as the break down of filamentous bacteria. In consequence, a better settle was achieved. The anoxic mixing stage, between the two settling stages, has been optimised to get the most advantageous mixing time and speed, 15 minutes and 300 rpm, respectively. This was in agreement with Mata et al. [29] who reduced the settling time by 20%, this giving a decrease in SVI from 325 ml/g to 67 ml/g. In the same vein, Guo et al. [7] achieved significant sludge settleability with anoxic feeding and recommended a mixing stage to improve settling. Mata et al. [29] found that SVI values decreased by reducing the settling time and allowing intermittent aeration to provide more air, this supporting the first reason for improving the settleability in the TSSBR.

The second reason for enhancing the settling performance in the TSSBR could be due to the minimisation of the anaerobic environment by breaking down the settling time from one hour into two stages: 15 minutes and 30 minutes, separated by a 15 minutes anoxic stage. This created a negative environment for filamentous bacteria leading to a halt in its growth and an enhanced settling performance in the TSSBR. This result is in agreement with Guo et al. [7] who reported that with low DO concentrations (0.5 mg/l), sludge settling declined (SVI> 200 ml/g). Liao et al. [42] found that flocculation ability improved when increasing the DO level from 1-2.5 mg/l to 3.5-5.5 mg/l, which also supports the second reason for improvement, decreasing the long settling stage and increasing the DO level by mixing (anoxic stage). DO level was monitored in both NOSBR and TSSBR. In the settling stage, DO was 3.2 mg/l in the NOSBR, while it was 4.3 mg/l in the TSSBR due to the anoxic mixing stage in the TSSBR.

This result is in agreement with Ozbek and Gayik [43], who stated that oxygen transfer to the bioreactor increases through stirring.

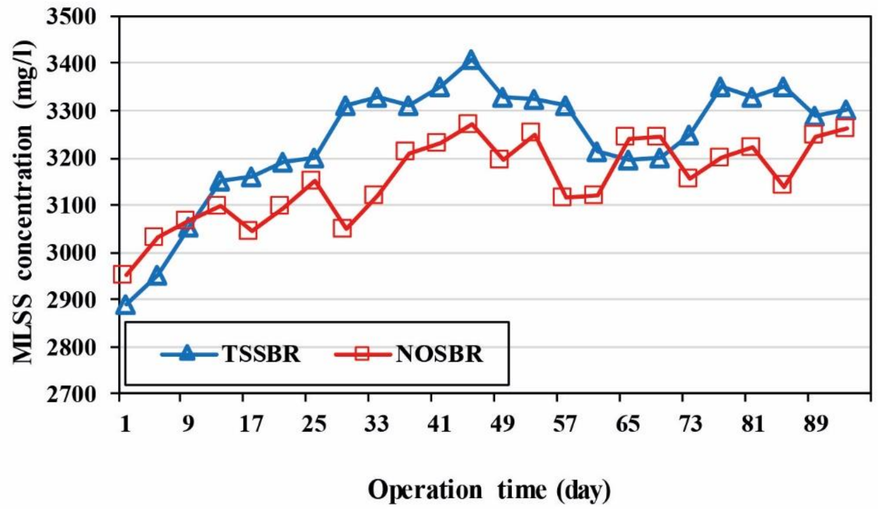

Fig. 7. MLSS concentration for NOSBR and TSSBR over the whole period of study.

3.3 Statistical analysis

Statistical analyses have been performed to assess the performance of the studied reactors, TSSBR and NOSBR, in terms of SVI and the removal of COD, NH_3-N, NO_3-N and NO_2-N. Three key parameters were investigated; the standard deviation, outliers and the normality (according to Kolmogorov-Smirnov test) of the obtained results. The standard deviation describes the amount of variation in the parameter under investigation; the smaller the standard deviation, the better the consistency and quality of the treatment process [44-46]. The presence of outliers, which could be defined as extreme observations, indicates a poor and unstable performance, while the normality of the obtained results enhances the ability to model treatment performance [47-49]. Additionally, the significance of the difference in performance of TSSBR and NOSBR has been assessed using the t-test.

The results obtained from the statistical analysis confirmed that the performance, in terms of SVI and the removal of COD, NH_3-N, NO_3-N and NO_2-N in the TSSBR, is more reliable and predictable than that of NOSBR. It can be clearly seen from the results (Table 3) that the standard deviation of the effluent SVI, NH_3-N, NO_3-N and NO_2-N from TSSBR, is much lower than the same effluents in NOSBR, which indicates that TSSBR has better consistency and quality of treatment. In terms of outliers, the results of the statistical analysis (Table 3), indicate that the performance of TSSBR is stable as it does not show any extreme readings in effluents SVI, NH_3-N, NO_3-N and NO_2-N. The performance of NOSBR was unstable over the studied period as it showed extreme effluent concentrations of both COD and NH_3-N. Finally, in terms of the normality of the obtained results, the Kolmogorov-Smirnov test (ρ of K-S test) indicated that the effluents of both TSSBR and NOSBR followed a normal distribution (ρ of K-S test > 0.05), except for NO_3-N from NOSBR, which showed a skewed distribution.

Finally, it should be noted that the calculated mean values of the removal of COD, NH_3-N, NO_3-N and NO_2-N, by TSSBR and NOSBR, confirmed the superior performance of TSSBR. It can be seen from Table 3 that the SVI value of TSSBR is smaller than that of NOSBR, indicating that TSSBR had better sludge settleability than NOSBR. In addition, the outcomes of t-test confirmed the superior performance of TSSBR, in terms of SVI and the removal of NO_3-N and NO_2-N, where the statistical significance of the t-test (ρ of the t-test) of these parameters were 0.000, 0.001, and 0.044, respectively. Although TSSBR achieved higher removal of COD and NH_3-N than NOSBR, the calculated value of ρ of the t-test was greater than 0.05, which indicates insignificance difference.

Table 3: Results of the statistical analysis

	TSSBR				NOSBR			
Parameter	Mean	Standard deviation	Outliers %	ρ of K-S test	Mean	Standard deviation	Outliers %	ρ of K-S test
SVI	39.36	10.26	0	0.210	54.86	21.14	0	0.200
COD	93.51	1.061	0	0.198	93.14	1.083	8.3	0.188
NH_3-N	89.24	3.42	0	0.200	76.66	5.31	8.3	0.200

NO_3-N	95.24	1.38	0	0.220	86.44	4.32	0	0.028
NO_2-N	95.99	1.18	0	0.178	87.32	2.13	0	0.169

3.4 pH, DO, ORP and temperature profiles

The monitoring of pH, DO, ORP and temperature for the TSSBR and NOSBR, is shown in Fig. 8. The pH, DO, ORP and temperature values at the end of the 5.5 h treatment cycle fluctuated between 6.5-8.5, 0.4-6 mg/l, -122 to198 mV and 7-11 ◦C, respectively. In the activated sludge process, DO is related to the aerobic stage, while pH and ORP are related to the anoxic and anaerobic stages. The microbial activity in the SBR system is responsible for the variation in the DO profile. Bacteria utilize the DO in the system to oxidize COD and ammonia. At the first stage of the treatment, the anoxic fill, the ORP profile decreases due to the denitrification occurring in the presence of a carbon source in the influent wastewater and anoxic environment [50]. In the react stage, the aerobic condition, the oxidation of COD begins; this is seen by the increased concentration of ammonia in Fig. 4. In this stage, the DO profile increased continuously, while the ORP profile decreased. This might be due to the high concentration of COD in the system. This finding is in agreement with Li and Irvin [51], who stated that during the anoxic period, ORP dropped to −104 mV under high COD conditions (1317 mg/L), while ORP was still as high as 178mV under low COD conditions (88 mg/L). by 160 minutes into the process, nitrification has started, this seen a decrease in ammonia and an increase in nitrite and nitrate concentrations, as shown in Fig. 4. At this stage, both DO and ORP profiles have increased dramatically [52]. Denitrification occurred at 225 minutes as shown in Fig. 4, identifiably by a decrease in nitrate concentrations. At this stage, the ORP profile increased due to denitrification [53], while the DO profile remained constant. At the settle stage, DO concentrations have decreased sharply towards the end of the treatment in the NOSBR, while in the TSSBR, the DO profile decreased at the first settle stage. Its values did not change after that because of the transfer of oxygen into the reactor in the anoxic mixing stage, its values decreasing again in the second settling stage. Based on these results, pH, DO and ORP are considered important parameters that can indicate different behaviours in COD and nitrogen removal.

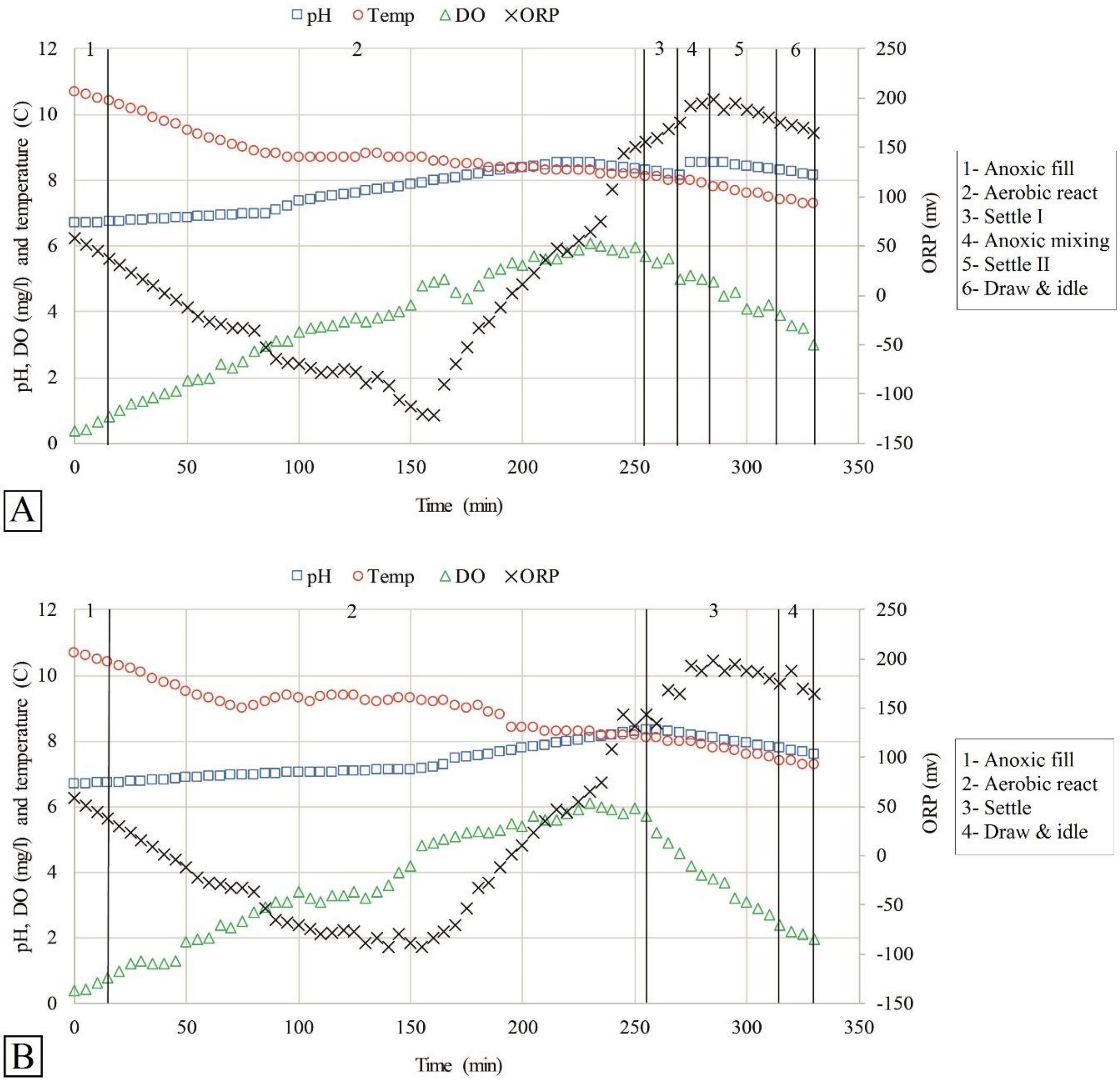

Fig. 8. pH, DO, temperature and ORP profiles for one treatment cycle of a) TSSBR, b) NOSBR.

Conclusion

The efficiency of COD and nitrogen compound removal, along with the settling performance of normal operating sequencing batch reactors, were determined and compared with the performance of a novel, two-stage, settling sequencing batch reactor to examine sludge settleability in the SBR as this is considered a major drawback for SBRs. The results obtained from this study showed that a TSSBR with a 5.5 h cycle time improved sludge settleability and enhanced nitrogen compounds removal efficiency, while COD removal efficiency for the NOSBR and TSSBR remained the same. The morphological characteristics of the sludge inside the TSSBR reactor, showed better and homogenous growth of filamentous bacteria in comparison to that in the NOSBR which showed overgrowth of filamentous bacteria. Finally, a significant linear relationship between the total filament length and SVI was found, this having a direct effect on sludge settleability.

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